Manganese and Compounds Reference Exposure Levels

1. Summary

Acute inhalation of high levels of manganese results in a nonspecific pulmonary edema, while chronic manganese inhalation leads to a characteristic neurotoxicity known as manganism with strong similarities to Parkinson's disease. Manganism is characterized by motor deficits (dystonia, altered gait, fine tremor, generalized rigidity) and may include psychiatric disturbances. At low manganese levels and in the absence of frank manganism, subtle deficits in cognitive and neurobehavioral functions have been reported in both adults and children. Neurodevelopmental deficits have been associated with early life exposure to excessive manganese and include impaired intellectual performance and behavioral disinhibition.

1.1 Manganese Acute REL

An acute REL for manganese was not developed at this time.

1.2 Manganese 8-Hour REL

Reference Exposure Level	**0.05 μg/m^3**
Critical effect(s)	Impairment of neurobehavioral function in humans
Hazard index target(s)	Nervous system

1.3 Manganese Chronic REL

Reference Exposure Level	**0.03 μg/m^3**
Critical effect(s)	Impairment of neurobehavioral function in humans
Hazard index target(s)	Nervous system

2. Physical and Chemical Properties

Table 2.1 Manganese and Manganese Species*

Molecular Formula	Synonyms	Molecular Weight	CAS Reg. No.
Mn	elemental manganese; colloidal manganese; cutaval	54.94 g/mol	7439-96-5
MnO	manganese oxide; manganese monoxide; manganosite	70.94 g/mol	1344-43-0
MnO_2	manganese dioxide; black manganese oxide	86.94 g/mol	1313-13-9
Mn_3O_4	manganese tetroxide; trimanganese tetraoxide; manganomanganic oxide	228.82 g/mol	1317-35-7
$MnCl_2$	manganese chloride; manganese dichloride; manganous chloride	125.84 g/mol	7773-01-5

Description	Lustrous, gray-pink metal (Mn); green (MnO), black (MnO_2) or pink ($MnCl_2$) crystals; brownish-black powder (Mn_3O_4)
Molecular formula	see Table 2.1
Molecular weight	see Table 2.1
Density (in g/cm^3)	7.21-7.4 (Mn – depending on allotropic form); 5.43-5.46 (MnO); 4.88 (Mn_3O_4); 2.977 @ 25°C ($MnCl_2$)
Boiling point	2095°C (Mn); not available (MnO); unknown (Mn_3O_4); 1190°C ($MnCl_2$)
Melting point	1246°C (Mn); 1839°C (MnO); 1567°C (Mn_3O_4); 650°C ($MnCl_2$) (CRC, 2005)
Vapor pressure	1 torr @ 1292°C (Mn); non-volatile at room temperature (Mn_3O_4); not available (MnO; $MnCl_2$)
Solubility	Sol. in dil. acids and aq. solns. of Na- or K-bicarbonate (Mn); sol. in NH_4Cl, insol. in H_2O (MnO); insol. in H_2O, HNO_3, or cold H_2SO_4 (MnO_2 -(Merck, 1976); insol. in H_2O, sol. in HCl (Mn_3O_4); 72.3 g/100 ml H_2O @ 25°C ($MnCl_2$)
Conversion factor	Not applicable (dusts or powders)

3. Occurrence and Major Uses

Metallic manganese is used in the manufacturing of steel, carbon steel, stainless steel, cast iron, and superalloys to increase hardness, stiffness, and strength (HSDB, 2006). Manganese chloride is used in dyeing, disinfecting, batteries, and as a paint drier and dietary supplement. Manganese oxide (MnO) is used in textile printing, ceramics, paints, colored glass, fertilizers, and as food additives. Manganese dioxide is used in batteries and may also be generated from the welding of manganese alloys. Manganese tetroxide may be generated in situations where other oxides of manganese are heated in air (NIOSH, 2005). The 2004 annual statewide emissions of manganese reported in the most recent California Toxics Inventory (CARB, 2005a) were estimated to be 1,055 tons. For 2002, the mean statewide ambient level was 31.5 ng/m^3.

4. Metabolism / Toxicokinetics

Manganese can enter the body by both the oral and inhalation routes. Dermal absorption of manganese is insignificant. Essential manganese is normally absorbed from the intestinal tract as part of the diet. It is estimated that 2 to 5% of ingested manganese is retained in the adult body (Andersen et al., 1999). Retention can be up to 41% in breast-fed infants, and 20% in formula-fed infants (Dorner et al., 1989). Manganese absorption is increased (along with iron absorption) when there is a deficiency of iron in the diet (Davis et al., 1992). Ascorbic acid, calcium and phosphorus also affect manganese utilization (ibid).

As part of the normal manganese homeostatic mechanism, high levels of dietary manganese diminish absorption from the intestinal tract. Manganese appears to be absorbed from the gut largely in the divalent form, with approximately 80% of it subsequently bound in plasma to ß$_1$-globulin and albumin (Foradori et al., 1967). These manganese-protein complexes are efficiently removed from the blood by the liver and returned to the gut in bile for elimination, thus establishing an entero-hepatic circuit for manganese. In the blood, unbound manganese may be converted by ceruloplasmin to the trivalent cation which is then bound by transferrin. Transferrin-manganese complexes are much less efficiently removed by the liver and thus survive first pass elimination to circulate throughout the body (Gibbons et al., 1976). In the brain, transferrin receptors in the capillary beds may mediate uptake in regions with efferents to the nucleus accumbans and the caudate putamen. Other mechanisms also appear to contribute to brain uptake of manganese including a divalent metal transporter (DMT-I), and a less well-defined non-saturable mechanism. From these sites, manganese is thought to move by neuronal transport to the pallidum, thalamic nuclei and substantia nigra; areas involved with motor control and movement (Aschner et al., 2005). While at normal plasma levels, manganese enters the brain mainly across the capillary epithelium, at elevated levels of manganese in the blood, transport across the choroid plexus becomes more prominent (Aschner, 2000).

Manganese exposure via the pulmonary route leads to more rapid absorption with higher efficiency, and with greater transfer to the brain compared with other routes (Drown et al., 1986; Roels et al., 1997). In experiments in rats, Roels *et al*. (1997) used

intratracheal instillation as a surrogate for inhalation for comparison with the oral route (gavage). Intratracheal instillation of $MnCl_2$ (1.22 mg/kg, once weekly for four weeks) raised the steady state manganese levels 68% in blood, 205% in the striatum, 48% in the cortex, and 27% in the cerebellum compared to controls. By gavage, a much higher dose of $MnCl_2$ (24.3 mg/kg) was required to achieve the same blood levels (68%). However, by this route, manganese levels in the striatum and cerebellum were not affected, and levels in the cortex were raised by only 22% (Table 4.1). In animals given a single intratracheal dose of $MnCl_2$ (1.22 mg/kg bw), blood manganese levels peaked within 30 min at 7,050 ng/100 ml. This was followed by a gradual decline but blood levels remained elevated over controls for at least 24 hours. By comparison, the single oral administration of 24.3 mg $MnCl_2$/kg bw resulted in a five-fold lower peak blood level of 1,660 ng/100 ml after one hour, followed by a return to control levels in 12 hours. Thus, compared to ingestion, inhalation of a relatively water soluble form of manganese leads to a rapid increase in blood levels that remain higher for longer, and result in higher brain manganese levels.

Table 4.1 Increase in Tissue Manganese by Route and Chemical Form

	Increase in Tissue Manganese (%)			
Chemical Form and Route	Blood	Striatum	Cortex	Cerebellum
$MnCl_2$ Intratracheal (1.2 mg/kg)	68	205	48	27
$MnCl_2$ Gavage (24.3 mg/kg)	68	0	22	0
MnO_2 Intratracheal (1.2 mg/kg)	41	48	34	31
MnO_2 Gavage (24.3 mg/kg)	0	0	0	0

Using the same exposure protocol with the less soluble MnO_2, intratracheal instillation raised manganese levels 41% in blood, 48% in striatum, 31% in cerebellum, and 34% in cortex. By contrast, neither blood nor brain levels were increased following oral exposure (Table 4.1). As with $MnCl_2$, Mn blood levels following intratracheal MnO_2 reached a higher peak value (1,760 ng Mn/100 ml; 200% increase) than that achieved after gavage (900 ng/100 ml; 27% increase). Blood levels rose more slowly than with $MnCl_2$, starting at 48 – 72 hr after intratracheal instillation and peaking at 168 hr. By gavage, blood levels rose gradually to peak at 144 hr (Roels *et al.*, (1997). In these studies, the solubility of the manganese complexes influenced the rate of absorption by either route, but in both cases inhalation resulted in substantially higher blood and brain levels.

In a further demonstration of the dependence of tissue distribution on oxidation state and route of exposure, Reaney *et al.* (2006) exposed rats to 0, 2, or 6 mg/kg Mn(III)-pyrophosphate or Mn(II)Cl_2 intraperitoneally (i.p.) for five weeks. Significantly higher blood manganese levels were seen with Mn(III) vs equimolar Mn(II). A dose-dependent increase in brain manganese was observed, with Mn(III) producing levels that were 25% higher than following Mn(II). This may be related to the higher blood levels of manganese achieved with Mn(III) vs Mn(II) via the i.p. route. Examination of the striatum, globus pallidus, thalamus and cerebral cortex by PIXE (an x-ray fluorescence technique) revealed no differences in the distribution of manganese across these brain

regions. There were, however, differences among regions in response to the concentration and oxidation state of the manganese. In the globus pallidus, the highest cumulative dose (90 mg/kg) of both forms of manganese increased GABA levels compared to controls. By contrast, dopamine levels in globus pallidus at this dose were increased by 60% with Mn(III), but decreased by 40% with Mn(II). The mechanism behind this differential effect is not clear but suggests that manganese oxidation states are important in manganese toxicity.

Drown and colleagues studied the distribution of soluble $^{54}MnCl_2$ and insoluble $^{54}Mn_3O_4$ after instillation into the rat lung (Drown et al., 1986). Initially the soluble form of manganese distributed more rapidly from the lung to the peripheral tissues than did the insoluble form. After two weeks the rates of distribution of the two forms became almost equal. Manganese (^{54}Mn) reached higher concentrations in the liver, kidney and gastrointestinal tissues, but persisted longer in the heart, brain and bone. The manganese was eliminated mainly in bile with very little elimination in urine.

For humans with occupational and/or environmental exposures, the main route of exposure is via inhalation. In both cases the manganese is usually in the form of particulates of various sizes. Manganese deposited in the lung can be absorbed directly into the blood stream, or can migrate (by mucociliary transport) into the upper respiratory tract and then be swallowed for possible absorption in the GI tract. In experimental animals it has been demonstrated that inhaled manganese may be transported via olfactory nerves directly to the brain following absorption from nasal passages (Brenneman et al., 2000; Dorman et al., 2002a; Elder et al., 2006). Neither pulmonary nor gastrointestinal absorption is required for this route of exposure, and the blood-brain barrier is bypassed. Evidence for absorption of particulate manganese oxide from the nose and transport to the brain was provided by Elder *et al.* (2006) in rats. Manganese concentrations in the olfactory bulb increased 3.5-fold following 12 days of intranasal instillation of ultrafine manganese oxide particles (3-8 nm) in both nares. With occlusion of the right nostril and instillation in the left naris, manganese accumulated almost exclusively in the left olfactory bulb. In this experimental paradigm, instillation of either the soluble manganese chloride or the insoluble manganese oxide particles (solubilization rate 1-1.5% per day) in the patent naris resulted in comparable levels of manganese in the ipsilateral side of the olfactory bulb. This, in conjunction with the observation that an increase in manganese in the olfactory bulb was detectable within 30 minutes of the instillation, suggests that particulate rather than dissolved manganese was the form transported to the brain. It is not clear how significant this route of exposure is in humans.

The major route of excretion of manganese is via bile, although a lesser amount is excreted via urine (Davis et al., 1993; ATSDR, 2000). That the liver maintains homeostasis of manganese can be seen by the fact that patients with cirrhosis of the liver accumulate abnormally high levels of manganese in their brains, especially in the globus pallidus (Rose et al., 1999). Similarly, rats that have a liver bypass also show high levels of manganese in the brain, especially in the globus pallidus (Rose *et al.*, 1999).

Neonatal humans do not excrete manganese for the first two to three weeks of life. The intestinal barrier to manganese absorption is also immature in premature and neonatal infants (Cawte, 1985).

5. Acute Toxicity of Manganese

Acute inhalation exposure to high levels of manganese as its oxides is associated with pulmonary edema and impaired function (Shiotsuka, 1984). However, a pulmonary inflammatory response is also associated with the inhalation of particulates in general and does not appear to be dependent on the manganese content. The very small body of literature on acute toxicity includes two animal experiments involving acute exposures by inhalation. One is a two-hour exposure of female CD-1 mice to manganese oxide (Mn_3O_4) aerosols (Adkins et al., 1980) that resulted in a NOAEL of 2.9 mg/m^3 based on respiratory effects (edema). The other is a 24 hr exposure of guinea pigs to 22 mg/m^3 MnO_2 (Bergstrom, 1977) that resulted in a NOAEL of 0.9 mg/m^3, corrected for one hour, again for respiratory effects (inflammatory reaction). However, no LOAEL was observed in either study, and no manganese acute inhalation studies were located that demonstrated a dose-response or evaluated other toxicological endpoints.

6. Chronic Toxicity of Manganese

6.1 Chronic Toxicity to Adult Humans

Exposure of humans to manganese by inhalation leads to a suite of neurological effects called "manganism" (Lucchini et al., 1999). Frank manganism is a progressive disease that involves symptoms similar to those of Parkinson's disease (ATSDR, 2000). Manganism is characterized by altered gait, fine tremor and occasionally psychiatric disturbances. The psychiatric disturbances are seldom seen in Parkinson's disease, although dementia sometimes occurs late in this disease. Despite their similarities, the symptoms of manganism and Parkinson's disease differ somewhat (Barbeau, 1984; Calne et al., 1994). Both manganism and Parkinson's disease involve generalized bradykinesia and widespread rigidity. However, tremor is less frequent and dystonia more frequent in manganism. Manganism is also distinguished by a propensity to fall backward, failure to achieve a sustained therapeutic response to levodopa, and failure to detect a reduction in fluorodopa uptake by positron emission tomography (PET) (Calne *et al.*, 1994). In Parkinsonism, the damage appears to be confined to the substantia nigra, whereas in manganism the damage is more widespread, involving other parts of the basal ganglia (Huang et al., 1998).

Manganese accumulates in certain brain structures, especially the extrapyramidal system. Structures rich in dopaminergic neurons show a heightened sensitivity to manganese toxicity. Within these tissues, manganese is found preferentially in mitochondria where it disrupts oxidative phosphorylation and mitochondrial function (Gavin et al., 1999). Cytochrome c, released from damaged mitochondria, leads to apoptosis and loss of neurons (Malecki, 2001). Trivalent manganese can promote the formation of reactive oxygen species (HaMai et al., 2001) that can cause oxidative stress, which in turn has been shown to lead to apoptosis of neurons in the rat brain (Dobson et al., 2003). While

individuals exposed to massive amounts of manganese show frank neurological symptoms as in the Groote Eylandt studies (Kilburn, 1987) and the industrial workers studies (ATSDR, 2000), individuals exposed to lesser amounts of manganese show more subtle neurological deficits in neurobehavioral tasks (Wennberg *et al*., 1992; Lucchini *et al*., 1999).

Adverse effects may occur at exposure levels to manganese that are too low to cause frank manganism. Lucchini and his co-workers studied a group of Italian ferroalloy workers who had been exposed to low levels of manganese dust by inhalation (Lucchini *et al*., 1999). These workers did not exhibit the frank signs of manganism, but they did exhibit subtler neurofunctional changes. The workers were exposed to a "current overall value" of 0.054 mg Mn per m^3 air at the time of the study. Earlier exposures were higher. In order to obtain a measure of cumulative exposure the investigators calculated a "cumulative exposure index" for each worker based on their exposure history in the factory. The cumulative exposure indices correlated positively with blood manganese levels. The workers were subjected to symptom questionnaires and neurobehavioral and neurophysiological testing for the purpose of finding whether neurological effects correlated with cumulative exposure. In multiple regression analyses, positive correlations were found between the log of the cumulative exposure index and the following tests of the Swedish Performance Evaluations System: finger tapping in the dominant ($R = 0.32$, $p = 0.01$) and non-dominant ($R = 0.32$, $p = 0.01$) hands, Symbol Digit ($R = 0.33$, $p = 0.01$) and Digit Span ($R = 0.44$, $p = 0.004$). The moderate but significant correlation coefficients reported in this study suggest that manganese is an important contributor to these effects but likely not the only one. In addition, these results demonstrate that subtle neurological changes are taking place in workers exposed to relatively low levels of manganese in the absence of frank manganism.

Male workers ($n = 92$, plus 101 matched controls) in an alkaline battery plant in Belgium exposed to manganese dioxide dust were the subjects of a cross-sectional epidemiological study (Roels et al., 1992). Total manganese concentrations, and manganese dust were measured in the workers' breathing zones with personal samplers. Lifetime integrated respirable dust levels (LIRD) ranged from 0.04 to 4.43 mg Mn/m^3 * year, with a geometric mean of 0.793 mg Mn/m^3 * year. The average age of control and exposed groups was 30 years with a mean manganese exposure time of 5.3 years (0.2 to 17.7 years) for the latter group. In exposed workers, the geometric mean levels of blood and urine manganese (corrected for creatinine) were significantly higher ($p < 0.001$) than in controls. The subjects were also evaluated for neurobehavioral function, lung function, and hematological parameters. There were no significant differences in respiratory symptoms between those exposed and controls, and hematological parameters were in the normal range for both groups. In neurobehavioral tests, significant decrements in performance were found in exposed workers on tests for visual reaction time ($p < 0.001$), five measures of eye-hand coordination ($p < 0.005$), and in two of three tests of hand tremor ($p < 0.03$). The LOAEL for this study, based on neurobehavioral effects, corresponds to 0.15 mg respirable manganese dust/m^3 (geometric mean of the LIRD divided by the average exposure time = 0.793 mg Mn/m^3 * year / 5.3 year = 0.15 mg Mn/m^3). This LOAEL was used to calculate the 8-hr and chronic RELs.

Another occupational study of lower exposures was done in Sweden (Wennberg et al., 1992). In this study workers had been exposed for a year or more to manganese dust at mean value concentrations of 0.18 mg/m^3 at one smelter, and 0.41 mg/m^3 at another. They were compared to workers at similar industrial plants without manganese exposure via a suite of neurological tests, including electroencephalogram, brainstem auditory evoked potential, event related auditory evoked potential, and diadochokinesometry (a test of the subject's ability to rotate a handle rapidly). Of these tests, the only one that produced significantly different results in the exposed subjects was the diadochokinesometry. The manganese-exposed workers were unable to rotate the handle as quickly as the control workers. This is interpreted as evidence of a "preclinical" effect of low-level manganese exposure.

A major study of non-industrial human exposures is the study of the natives of Groote Eylandt, a large island off the coast of Australia. The inhabitants of this island are Australian Aborigines. The island is so rich in manganese that the environment has been described as a "manganese ecology" (Kilburn, 1987). The inhabitants are exposed by virtually all routes of exposure, but especially by ingestion of food and water high in manganese. Kilburn studied the natives of Groote Eylandt and compared them to a control group of Australian Aborigines living in another part of Australia. This paper does not quantitate the manganese exposures or body levels of manganese in the study population, and it would be difficult to quantitate exposures in this complex environmental situation. Kilburn reports certain congenital abnormalities, such as deformations of the foot (talipes equinovarus), closed anus (imperforate anus), and anorectal malformations, and neurobehavioral problems, including progressive muscle wasting (amyotrophy) and failure of muscle coordination (ataxia), that apparently occur with greater frequency in the islanders than in the control groups, but these could also be due to genetic factors present in this small population. Indeed all of the problems were seen in just two pedigrees. A likely interpretation would be that the adverse health effects observed reflect gene-environment interactions.

6.2 Chronic Toxicity to Infants and Children

Manganese is an essential nutrient, but it has toxic effects if exposure is excessive or prolonged, especially if exposure is by the inhalation route. Infants and children are expected to be more susceptible than adults to the toxic effects of elevated manganese because they absorb greater amounts of manganese in their gastrointestinal tract, the infant liver has not yet developed the homeostatic mechanisms to maintain safe levels of manganese in the blood and target tissues (especially the brain), and the blood-brain barrier is incompletely formed. Indeed, this susceptibility is evident in a study by Wasserman *et al*. (2006) of 10-year old children (n = 142) in Bangladesh exposed to manganese in drinking water (< 200, 200-499, 500-999, >1,000 μg/l). Comparing the lowest and highest dose groups (< 200 vs > 1,000 μg/l), significant decrements in intellectual function at 9.5-10.5 years of age were revealed in scores on the Wechsler Intelligence Scale for Children-III with increasing daily intake of manganese (full scale, $p < 0.0001$; performance, $p < 0.0001$; verbal, $p < 0.02$). The scores of children with

intermediate manganese exposures were also lower than those of the lowest dose group, but not significantly so. In this study, confounding by co-exposure to arsenic was limited by including only children whose drinking water contained <10 µg As/l. Scores were adjusted for maternal education and intelligence, house type, television, child height and head circumference. Blood levels of manganese, arsenic and lead were also determined and added to the core model. In this case, only blood lead was correlated with decreased intellectual performance. However, in a simultaneous analysis of water manganese, water arsenic, and blood lead, the negative association between manganese water levels and intellectual function test scores remained (Full-Scale β = -4.56, $p < 0.01$; Performance β = -3.82, $p < 0.01$).

A number of studies have reported correlations between early life exposure to excessive manganese and symptoms of impaired neurodevelopment as revealed on neurobehavioral tests and in poorer academic performance. In a prospective study of the neurobehavioral effects of *in utero* exposure to manganese, Takser *et al.* (2003) reported an inverse correlation between cord blood manganese at birth and three subscales of psychomotor development (McCarthy scales of children's abilities) measured at three years of age (n = 126): attention (partial r = -0.33, $p < 0.01$), nonverbal memory (partial r = -0.28, $p < 0.01$) and hand skills (partial r = -0.22, $p < 0.05$). The adverse effects of manganese on neurodevelopment in these children persisted after adjustment for gender and maternal education, although the effects of manganese on hand skills were only observed in boys. Similarly, Collip et al. (1983) used a battery of tests, including cognitive and projective tests, psycho-educational evaluation, speech, language and hearing evaluations, and social services evaluations, to identify children who were hyperkinetic and exhibited learning disabilities. In comparison with normal children of the same age, significantly elevated levels of hair manganese (0.434 µg/g; measured at 8 years of age) were reported in children with learning disabilities and hyperactivity compared with normal children (0.268 µg/g) ($p<0.05$).   An association between poorer performance in school and elevated hair manganese (1.242 µg/g) has also been observed among children in China compared with children with more normal manganese levels (Zhang et al., 1995).

The uptake of metals into developing teeth provides a record of gestational exposure to manganese. In multiple regression analyses, after controlling for lead, high levels of manganese incorporated into teeth during the 20th week of gestation were positively correlated with behavioral disinhibition at 36 months of age (R = 0.48, $p < 0.01$) and, at 54 months, with impulsive errors on the Mirsky Continuous Performance Test (R = 0.48, $p < 0.01$) and the Children's Stroop Test (R = 0.38, $p < 0.01$).   Positive correlations with manganese were also seen in ratings made by both parents and teachers of externalizing and attention problems on the Child Behavior Checklist in the 1st (R = 0.40 – 0.47, $p < 0.05$) and 3rd grades (R = 0.38 – 0.48, $p < 0.05$), and in the 3rd grade with the teachers' ratings on the Disruptive Behavior Disorders Scale (R = 0.44, $p < 0.05$), ADHD (R = 0.48, $p <0.01$), and hyperactivity – impulsivity (R = 0.55, $p < 0.01$). In contrast, manganese levels in tooth enamel formed in the 62-64th week of gestation (i.e. postnatally) were correlated only with teachers' reports of externalizing behaviors in the 1st (R= 0.40, $p < 0.05$) and 3rd grades (R = 0.57, $p < 0.01$). It thus appears that high prenatal manganese exposure may adversely affect behaviors expressed postnatally.

There was, however, no correlation between tooth manganese and cognitive ability as measured on the Woodcock-Johnson Psycho-Educational Battery (Ericson et al., 2006).

Subtle neurobehavioral effects were seen in a case report of a 10-year old boy exposed for five years to elevated manganese in the family's drinking water (Woolf et al., 2002). The boy's hair manganese was high (3,091 ppb vs normal reference <260 ppb), as was that of his 16 year-old brother (1,988 ppb). Neuropsychological tests on the 10 year-old revealed intact global cognitive skills but striking deficits in visual and verbal memory (<20th percentile in the Wide Range Assessment of Visual-motor Abilities). No obvious neurobehavioral problems were noted for either the parents or the older sibling.

6.2.1   Potential for Differential Effects in Children

Infants and children may be more susceptible than adults to manganese toxicity for the following reasons:

1. Newborns absorb more manganese from the gastrointestinal tract than do adults.
2. The liver of newborns has not yet developed the ability to maintain safe levels of manganese in the bloodstream and brain tissues by excreting excess manganese in the bile, i.e. homeostasis of manganese has not yet developed.
3. Some infant formulas and foods are high in manganese.
4. The newborn's brain is still developing and the blood-brain barrier is not completely formed.
5. Modeling of the inhalation dosimetry of particles (0.001-10 µm), comparing neonates (3 mo) and adults, in four regions of the respiratory tract (extra-thoracic, tracheo-upper bronchi, bronchiolar, pulmonary), suggests that differences in the dose per unit surface area between neonates and adults are dependent on particle size and respiratory tract region (Ginsberg et al., 2005). In addition, infants and young children experience overall higher deposition of particles than adults.

6.3   Animal Studies of Chronic Toxicity

Animal studies of the toxic effects of chronic manganese exposure have focused on altered neurobehavior and the effects of manganese on the associated brain structures. These studies indicate that differences in age at exposure, route, and chemical form of the metal are critical to the distribution of manganese, and the type and extent of the adverse effects.

The relative sensitivity of neonatal and adult CD rats to manganese-induced neurotoxicity was studied by administering manganese dichloride orally to rats at doses of 0, 25 and 50 mg/kg per day (Dorman et al., 2000). Adults and pups were dosed for 21 consecutive days, and then were evaluated with behavioral tests such as pulse elicited startle response amplitude, and in terms of manganese levels in striatum, hippocampus, hindbrain and cortex. Neonatal rats exposed at the highest level of manganese showed a statistically

significant increase in amplitude of acoustic startle response. They also showed increases in brain levels of manganese. The results suggest that neonates may be at greater risk for manganese-induced neurotoxicity when compared to adults receiving high oral levels of manganese. The authors state that there are known pharmacokinetic processes that may relate to the increase in brain manganese concentration in neonatal rats including increased manganese absorption from the juvenile gastrointestinal tract, an incompletely formed blood-brain barrier, and a virtual absence of excretory mechanisms until weaning.

Neurobehavioral effects may be preceded by changes in brain chemistry. Such changes were studied in four female rhesus monkeys exposed in an inhalation chamber to 30 mg/m^3 respirable manganese dust for five h/d, five d/wk (Bird et al., 1984). After two years the animals were sacrificed and compared to unexposed controls. The exposed monkeys showed decreased dopamine in the caudate and globus pallidus, as well as a 60 to 80% increase in manganese levels in the basal ganglia of the brain. However, the exposed monkeys did not exhibit any of the movement disorders that are characteristic of Parkinson's disease.

The distribution of manganese in primate brain, and its neurobehavioral and cognitive effects in Cynomologus macaques following weekly intravenous injection of $MgSO_4$ (10-15 or 3.26-4.89 mg/kg) for 39 weeks was investigated by Guilarte and associates. Neurobehavior, as rated on a modified Parkinsonian symptoms scale, activity levels measured with an activity monitor, and fine motor skills, assessed as the number of errors while trying to retrieve objects from wells of different sizes, all showed significant decrements ($p < 0.05$) at the end of the experiment compared with baseline (Guilarte et al., 2006a). Over this same period, stereotypical or compulsive-like behaviors, such as licking/biting fingers and grooming significantly increased in frequency with manganese exposure ($p < 0.01$) (Schneider et al., 2006). Imaging studies were performed at 128 days and 157 days after the start of manganese exposure, and included T-1 weighted magnetic resonance imaging (MRI), magnetic resonance spectroscopy (H-MRS) and positron emission tomography (PET). As assessed by PET, manganese decreased the ability of amphetamine to stimulate dopamine release in the striatum, apparently without the loss of dopaminergic terminals. The authors speculate that the inhibition of dopamine release may alter the excitability of nigrostriatal dopaminergic neurons and/or may alter dopamine compartmentalization. The former case may contribute to the behavioral symptoms while, in the latter case, the probability of dopamine oxidation and consequent neuronal damage may be increased (Guilarte et al., 2006a).   Neuronal loss or dysfunction in these monkeys was suggested by a change in brain metabolites with chronic manganese exposure. Specifically, significant decreases in the N-acetylaspartate:creatinine ratio in parietal cortex ($p = 0.028$), and a near significant ($p = 0.055$) decrease in the white matter were observed.

6.4 Dietary Exposure to Manganese

Newborns and infants may be exposed to more manganese in their diets than are adults. Infant formulas based on cow's milk have about 16 times more manganese than human milk (Dorner et al., 1989). Soy based formulas have even higher levels of manganese – about 40 times the manganese of human milk (Tran et al., 2002a; Tran et al., 2002b).

Formula usage can lead to significantly elevated body burdens of manganese. For example, the hair manganese in normal infants at birth was reported to be 0.19 µg/g hair and, in breast-fed infants, increased to 0.330 µg/g at four months of age. By comparison, hair manganese levels in infants on a formula diet reached 0.965 µg/g at six weeks of age, and 0.685 µg/g at four months (Collipp et al., 1983). In addition, infants can have a less varied diet than adults and may consume more of certain foods that are high in manganese (e.g., sweet potatoes, 2.6 mg/cup; spinach, 1.8 mg/cup; oatmeal, 1.4 mg/cup; (NWU, 2006)).

6.5 Nutritional Requirement

Manganese is an essential nutrient involved in the formation of bone, and in amino acid, cholesterol, and carbohydrate metabolism (FNB, 2004). It is required in a number of metalloenzymes, including arginase, glutamine synthetase, phosphoenolpyruvate decarboxylase, and superoxide dismutase (FNB, 2004). Levels of manganese in adult tissues are maintained at stable levels by homeostatic mechanisms that involve regulation of both uptake and excretion (ATSDR, 2000). Manganese homeostasis is not maintained in newborn infants, and it is not clear how long it takes for it to develop (FNB, 2004); homeostasis in mice takes 17 to 18 days to become effective (Fechter, 1999). Rat pups born to manganese-exposed mothers (dosed with 2000 ppm Mn in drinking water throughout pregnancy and for 11 days of lactation) have seven times the manganese (whole body) as controls (Kostial et al., 2005). By weaning (11 days after birth) the manganese concentration in both groups is virtually the same, indicating that in rat pups manganese homeostasis may begin shortly after birth and become effective by weaning (Kostial *et al*., 2005).

Adequate intakes (AI) of manganese have been established by the Food and Nutrition Board of the Institute of Medicine (FNB, 2004). They are given in Table 6.5.1 below. This table also contains tolerable upper intake levels (UL) for manganese consumption. It is of note that in many cases the UL is not very far above the AI level. For children one to three years of age the UL is less than twice the AI.

The AI for infants 0 to 6 months was set based on the amount of manganese in human milk and the average amount of milk consumed. There are no reports of nursing infants showing any symptoms of manganese deficiency (FNB, 2004). The AI for infants 7 to 12 months of age is based on the manganese content of a typical diet including human milk and other foods. This AI is much higher than the one for infants 0 to 6 months because the manganese content of other foods is generally much higher than the manganese content of human milk (FNB, 2004).

Table 6.5.1 Adequate Intakes and Tolerable Upper Intake Levels for Manganese for Different Age Groups

Group	Adequate Intake (AI) (mg/day)	Tolerable Upper Intake Level (UL) (mg/day)
Infants, 0-6 months	0.003	"not possible to establish"
Infants, 7-12 months	0.6	"not possible to establish"
Children, 1-3 years	1.2	2
Children, 4-8 years	1.5	3
Boys, 9-13 years	1.9	6
Boys, 14-18 years	2.2	9
Girls, 9-13 years	1.6	6
Girls, 14-18 years	1.6	9
Men, 19 to >70 years	2.3	11
Women, 19 to >70 years	1.8	11
Pregnant women, 14-18 yrs	2	9
Pregnant women, 19-50 yrs	2	11
Lactating mothers, 14-18 years	2.6	9
Lactating mothers, 19-50 years	2.6	11

7. Developmental and Reproductive Toxicity

While data are scarce on the developmental effects of perinatal manganese exposure in humans, rats exposed to supplemental manganese (50, 250, 500 µg/day) beginning at birth show decreased dopamine in the striatum and poorer performance on behavioral tests (Tran et al., 2002b). In children on long-term parenteral nutrition resulting in blood manganese levels of 615-1840 nmol/l (vs reference range of 72-210 nmol/l), elevated manganese levels have been seen in globus pallidus and subthalamic nuclei (Fell et al., 1996), suggesting an enhanced potential for neurological damage. This is consistent with the decrements in intellectual function in children exposed to manganese in drinking water reported by Wasserman et al. (2006).

The effects of manganese on reproduction in humans have been reported in epidemiological studies of workers with occupational exposure to manganese. The results have been mixed with Gennart et al. (1992) reporting no effect on fertility among workers exposed to a median manganese dust level of 0.71 mg/m^3, while those exposed to 0.07-8.61 mg/m^3 (geometric mean 0.94 mg/m^3) in a study by Lauwerys et al. (1985) sired a statistically significant lower number of children during the period of paternal exposure. However, workers in the Gennart et al. study were exposed to the relatively insoluble manganese oxide and had mean urine manganese levels of 0.82 µg/g creatinine. By comparison, the workers in the study by Lauwerys et al. were exposed to the more soluble manganese salts in addition to the oxide, and had mean urinary manganese levels of 4.37 µg/g creatinine. Thus the differences in the effects of manganese on reproduction

reported in these two studies may be due to the significant differences in manganese exposures.

Adverse changes in reproductive parameters and behaviors have been seen in studies of rodents exposed to high levels of manganese. In immature female rats (23 days old), manganese (1-25 µg $MnCl_2$) introduced into the third ventricle of the brain significantly and dose-dependently stimulated the release of leutinizing hormone (LH). This effect was apparently at the level of the hypothalamus as pretreatment with the LH releasing hormone (LHRH) receptor antagonist, acycline, prior to manganese exposure blocked the release of LH (Pine et al., 2005). These authors further reported that serum LH, follicle stimulating hormone, and estradiol were all elevated by 29 days of age in rats that had received $MnCl_2$ by gavage starting on postnatal day 12. In these animals manganese altered the timing of reproductive events resulting in a significantly ($p<0.001$) earlier onset of puberty as measured by vaginal opening.

In adult male rats, exposure to 1,000 ppm manganese sulfate in drinking water for 12 weeks significantly suppressed sexual performance compared with controls as measured by prolonged ejaculatory latencies ($p < 0.001$), and increased post-ejaculatory intervals ($p < 0.05$). Displays of aggressive behaviors (lateralizations, boxing bouts and fights with stud males) were also reduced ($p < 0.001$). The extent to which the altered behaviors represent neurological effects versus effects on testes and androgen production is not clear. However, among females mated to the manganese-treated males, the total number of resorptions was significantly increased ($p < 0.025$), suggesting a testicular effect. This is supported by a significant ($p < 0.001$) reduction in absolute and relative testes weights, and absolute seminal vesicle weights among manganese-exposed males (Bataineh et al., 1998). An effect of manganese on male reproductive organs was also investigated in mice following 43 days of oral manganese acetate (7.5 – 30 mg/kg/d) (Ponnapakkam et al., 2003). Unlike the study with rats above, there was no significant change in testicular weight or pathology with manganese exposure in the mice. Nor was there evidence of abnormal mating behavior. However, epididymal weights were significantly lower ($p < 0.05$) and there was a significant ($p < 0.001$) dose-dependent decrease in sperm number and motility.

The available data suggest that manganese is a reproductive toxicant in animals (both males and females) albeit at relatively high doses. Neurobehavioral toxicity manifests at levels encountered in the environment (Wasserman *et al*., 2006). Whether this decrement in intellectual function represents a true developmental effect with permanent consequences is not clear.

8. Derivation of Reference Exposure Levels

The determination of safe exposure levels to manganese is complicated by its status as an essential nutrient. However, as described above, inhalation of manganese results in a qualitatively and quantitatively different exposure compared to oral intake, with inhalation resulting in more rapid uptake and higher blood and brain levels. While dietary manganese levels moderate intestinal absorption of manganese, there appears to be no affect of dietary manganese on the pharmacokinetics of inhaled manganese (Dorman et al., 2001; Dorman et al., 2002b). To provide perspective on potential manganese exposure from inhalation relative to the suggested upper limits for age-dependent dietary intake, Figure 8.1 below shows the amount of manganese children of various ages and lactating mothers (19-50 yr old) would inhale if they were exposed to the manganese levels reported in the Roels *et al* (1992) occupational study (0.215 mg/m^3) compared with the recommended adequate intakes and upper limits listed in Table 6.5.1. The study by Roels is used in the development of the RELs below, and the value of 0.215 mg/m^3 was selected from that study as it represents a real-world mean level of exposure that is associated with neurotoxicity in adults. The figure presents applied doses, not the actual amount absorbed since it is not known if humans absorb manganese more efficiently from the lungs than from the gut, as was shown to be the case for rats (Roels *et al*., 1997). However, the graph suggests that for infants and children receiving adequate dietary manganese, additional exposure to manganese by inhalation presents a greater risk for manganese overdose than it does for adults at a given air concentration. Further, evidence of neurodevelopmental toxicity in animals and humans underscores increased sensitivity in children relative to adults. This enhanced risk among infants and children will be addressed in the development of the RELs below.

Figure 8.1 Manganese Dose from Inhalation and Diet by Age
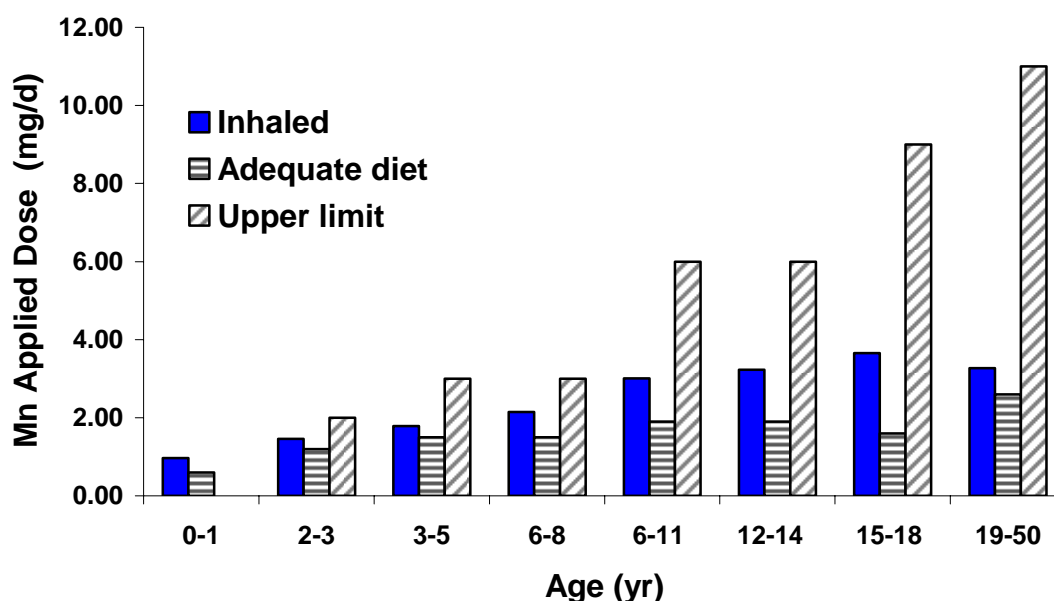
8.1 Manganese Acute Reference Exposure Level

Acute Reference Exposure Levels (RELs) are levels at which intermittent one-hour exposures are not expected to result in adverse health effects (see Section 5 of the Technical Support Document (TSD)). Pulmonary damage and inflammation are the principal endpoints associated with acute inhalation exposure to manganese. However, at present the database is insufficient to allow the development of an acute REL for manganese based on inhalation studies. No studies were located that reported dose-response data for acute inhalation exposures, nor was it possible to determine both LOAELs and NOAELs from the available data.

8.2 Manganese 8-Hour Reference Exposure Level

Study	Roels *et al.*, 1992
Study population	102 workers in a battery plant
Exposure method	Inhalation of workplace air
Exposure continuity	
Exposure duration	8 hr/day, 0.2-17.7 yr (mean 5.3 yr)
Critical effects	Impaired neurobehavior: visual reaction time, eye-hand coordination, hand steadiness
LOAEL	0.15 mg/m^3
NOAEL	not observed
Benchmark concentration	not derived
Time-adjusted exposure	0.107 mg/m^3 (0.15 mg/m^3 x 5/7)
LOAEL uncertainty factor (UF$_L$)	6 (default, no NOAEL, mild effect)
Subchronic uncertainty factor (UFs)	√10 (default 8-12% of lifetime)
Interspecies Uncertainty Factor	
Toxicokinetic (UF$_{A-k}$)	1 (default: human study)
Toxicodynamic (UF$_{A-d}$)	1 (default: human study)
Intraspecies Uncertainty Factor	
Toxicokinetic (UF$_{H-k}$)	10 (greater lung deposition in children)
Toxicodynamic (UF$_{H-d}$)	10 (greater susceptibility of children to neurotoxicity)
Cumulative uncertainty factor	2000
Reference Exposure Level	**0.05 μg/m^3**

The 8-hour Reference Exposure Level is a concentration at or below which adverse noncancer health effects would not be anticipated for repeated 8-hour exposures (see Section 6 in the TSD).

The proposed 8-hr REL for manganese is 0.05 μg/m^3 based on impairment of neurobehavioral function in humans in the occupational study of Roels et al. (1992) described in Section 6.1. This study yielded an average occupational exposure for the exposed group (LOAEL, no NOAEL observed) of 0.15 mg Mn/m^3 corresponding to a time-adjusted concentration of 0.107 mg/m^3 (based on an 8 hour TWA occupational exposure for 5 days/week).

A cumulative UF of 2,000 was used, comprising a 6 for the LOAEL uncertainty factor associated with mild effects (see Section 4.4.5 of the TSD), $\sqrt{10}$ for subchronic to chronic conversion (average exposure duration = 5.3 yr; Section 4.4.6 of the TSD), and 10 each for intraspecies toxicokinetic (UF_{H-k}) and toxicodynamic (UF_{H-d}) uncertainty. This REL is based on healthy adult male workers with adjustments for the potentially greater susceptibility of children. The intraspecies UF_{H-k} of 10 was chosen in part to reflect the 3-4-fold greater deposition of inhaled particulates in the 1-10 μm size range in the lungs of neonates relative to adults exposed to similar particulate levels in ambient air (Ginsberg et al., 2005). In addition, based on studies with neonatal and adult rats (Dorman et al., 2005), neonates accumulate higher levels of manganese in the brain more quickly than do adults with similar exposures.

A UF_{H-d} of 10 is used to address the expectation that the still-developing brain of newborn and infant children is more sensitive to the effects of manganese and that injuries to the nervous system during development are anticipated to have lasting effects.

8.3   Manganese Chronic Reference Exposure Level

Study	Roels et al., 1992
Study population	102 workers in a battery plant
Exposure method	Inhalation of workplace air
Exposure continuity	
Exposure duration	8 hr/day, 0.2-17.7 yr (mean 5.3 yr)
Critical effects	Impaired neurobehavior: visual reaction time, eye-hand coordination, hand steadiness
LOAEL	0.15 mg/m^3
NOAEL	not observed
Benchmark concentration	not derived
Time-adjusted exposure	0.054 mg/m^3 (0.15 mg/m^3 x 10/20 x 5/7)
LOAEL uncertainty factor (UF$_L$)	6 (default, no NOAEL, mild effect)
Subchronic uncertainty factor (UFs)	$\sqrt{10}$ (default 8-12% of lifetime)
Interspecies Uncertainty Factor	
Toxicokinetic (UF$_{A-k}$)	1 (default: human study)
Toxicodynamic (UF$_{A-d}$)	1 (default: human study)
Intraspecies Uncertainty Factor	
Toxicokinetic (UF$_{H-k}$)	10 (greater lung deposition in children)
Toxicodynamic (UF$_{H-d}$)	10 (greater susceptibility of children to neurotoxicity)
Cumulative uncertainty factor	2000
Reference Exposure Level	**0.03 μg/m^3**

The chronic Reference Exposure Level is a concentration at which adverse noncancer health effects would not be expected from chronic exposures (see Section 7 in the Technical Support Document).

The proposed chronic REL for manganese is 0.03 µg/m^3, based on impairment of neurobehavioral function in humans in the occupational study of Roels et al. (1992). This study yielded an average occupational exposure for the exposed group (LOAEL, no NOAEL observed) of 0.15 mg Mn/m^3 corresponding to a time-adjusted concentration of 0.054 mg/m^3 (based on an 8 hour TWA occupational exposure to 10 m^3 manganese contaminated air per day out of 20 m^3 total air inhaled per day over 5 days/week).

A cumulative UF of 2,000 was used, comprising a 6 for the LOAEL uncertainty factor associated with mild effects (see Section 4.4.5 of the TSD), 3 for subchronic to chronic conversion (average exposure duration = 5.3 yr; Section 4.4.6 of the TSD), and 10 each for intraspecies toxicokinetic (UF$_{H-k}$) and toxicodynamic (UF$_{H-d}$) uncertainty. This REL is based on healthy adult male workers with adjustments for the potentially greater susceptibility of children. The intraspecies UF$_{H-k}$ of 10 was chosen in part to reflect the 3-4-fold greater deposition of inhaled particulates in the 1-10 µm range in the lungs of neonates relative to adults exposed to similar particulate levels in ambient air (Ginsberg et al., 2005). In addition, based on studies with neonatal and adult rats (Dorman et al., 2005), neonates accumulate higher levels of manganese in the brain more quickly than do adults with similar exposures.

A UF$_{H-d}$ of 10 is used to address the expectation that the still-developing brain of newborn and infant children is more sensitive to the effects of manganese and that injuries to the nervous system during development are anticipated to have lasting effects. This REL was developed with specific consideration of the potentially greater susceptibility of children to manganese neurotoxicity. For comparison, the RfC for chronic manganese inhalation developed by the US EPA is 0.05 µg/m^3 (U.S.EPA, 1993) also based on Roels et al. (1992).

8.4 Manganese as a Toxic Air Contaminant

In view of the potential for high exposure coupled with a lower ability to regulate manganese, and enhanced neurodevelopmental susceptibility leading to differential impacts in infants and children identified in Section 6.2.1, OEHHA recommends that manganese be identified as a toxic air contaminant which may disproportionately impact children pursuant to Health and Safety Code, Section 39669.5(c).

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